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JOURNAL ARTICLE

INFLUENCE OF SEDIMENT IN COPPER TOXICITY TESTS WITH THE POLYCHAETE *NEANTHES ARENACEODENTATA*

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Abstract—Adult male *Neanthes arenaceodentata* were exposed to measured concentrations of 0.04, 0.06, 0.10, 0.16 and 0.26 mg l^{-1} Cu in a continuous-flow bioassay system, with and without a clean sand, to assess the influence of sand on Cu induced mortality. The presence of sand did affect the results. The 28-day LC50 was lower for polychaetes exposed without sand than those with sand, 0.044 and 0.10 mg l^{-1} Cu, respectively. The reasons for this difference are discussed.

INTRODUCTION

In recent years, the trend in conducting bioassays has been to use a greater variety of species as test organisms and to perform these tests in more natural conditions. Because of the importance of polychaetes in marine environments, investigators have started using this group of animals in bioassays. Most polychaete bioassays have been conducted in static systems without sediment (Raymont & Shields, 1963; Bellan *et al.*, 1972; Reish *et al.*, 1976; Rossi *et al.*, 1976; Jones *et al.*, 1976; and others). The section on polychaete bioassays in the Standard Methods for the Examination of Water and Wastewater, 14th Edition, states "It has not been found necessary to provide a substrate in the test containers...". Polychaete bioassays can be conducted without sediment and the survival of the control animals will be acceptable. However, it is well known that some toxicants, particularly heavy metals, interact with the sediment. By conducting an assay without sediment, the interaction of the polychaete, the toxicant, and the sediment is ignored. Jackim *et al.* (1977) found that the presence of sediments (sand and mud) depressed the uptake of cadmium in the soft shell clam, *Mya arenaria*.

In order to simulate more natural conditions to conduct polychaete bioassays, we designed a continuous-flow system to which sediment could be added. We used this system to test the effect of the presence and absence of a sediment on the mortality of the polychaete *Neanthes arenaceodentata* when copper was added to the sea water. The sediment used was a clean beach sand that had been treated in a muffle furnace to remove the organic material.

MATERIALS AND METHODS

Adult *N. arenaceodentata* were exposed to five concentrations of Cu in the seawater plus a control, each concentration with and without sand. Exposures were made in a continuous-flow system and lasted for 28 days. Observations of mortality were made throughout the experiment. At the end of the experiment, sand samples and live worms were analyzed for Cu.

Neanthes arenaceodentata used in this experiment were raised in the laboratory from specimens originally cultured by Donald Reish, California State University, Long Beach. This species forms numerous mucous-lined burrows throughout the sediment. The worms move freely through these burrows and come to the surface when food is added. They circulate water through the burrow by undulatory body movements.

The exposure system was modified from the system described by Buchanan, Myers & Caldwell (1975). Ten Nalgene* cups (70 × 62 mm diameter) were placed in each of six Plexiglass boxes (33.6 × 25 × 12.5 cm). Each cup had two holes, 22 mm in diameter, positioned 180° from each other. The holes were located 1.3 cm from the bottom of the cup and covered with Nytex* screen (210 μm mesh). The boxes had an inflow of water (20 ml min^{-1}) at one end and a siphon at the opposite end. The siphon, which was activated about every 3 h and discharged water at a rate of 200 ml min^{-1}, provided a fluctuating water level (1.7–6.5 cm) to improve the exchange of water in the cups.

Toxicant was metered into the inflow water by a peristaltic pump. We prepared the stock solutions by adding the appropriate amount of atomic absorption reference solution (10,000 ppm Cu in dilute nitric acid) to deionized water. Nominal Cu concentrations in the exposure boxes were 0.03, 0.06, 0.11, 0.18 and 0.32 mg l^{-1}. We measured the actual concentration of Cu in the stock solutions and exposure boxes twice weekly using a Perkin–Elmer 403 atomic absorption spectrophotometer. Sea water samples were taken from the control box weekly to monitor the background Cu concentration. The Cu from each control sample was concentrated and measured by flame atomic absorption analysis (Davey & Soper, 1975). The seawater used for the experiment was filtered (15 μm) Narragansett Bay water with a salinity of 31 ± 1‰ and temperature of 17 ± 1°C.

Clean beach sand from lower Narragansett Bay was sieved to a grain size of 300–420 μm, rinsed in deionized water, and baked in a muffle furnace at 500°C for 12 h. Analysis showed that the total organic carbon content of the muffled sand was 0.18 mg g^{-1}. We added 50 g (dry wt) of sand to five of the cups in each box to a depth of 1 cm. Adult male *N. arenaceodentata* were randomly distributed to the cups until there were two worms per cup.

*Trade names mentioned do not imply endorsement by the Environmental Protection Agency.

A glass rod, 7 mm in diameter, 60 mm long, was placed in each cup without sand to partition the space in the cups. This minimized confrontation between the worms, which demonstrate a fighting behavior (Reish, 1957). Twice a week during the experiment, the worms were fed *Enteromorpha* sp. (10 mg dry wt cup^{-1}).

The experiment was checked for mortalities daily for the first week and twice weekly for the remaining 3 weeks. Dead animals were removed and discarded. Worms that died in the cups containing sand were always found on the sediment surface. LC50 values were calculated graphically (Am. Publ. Hlth Ass., 1975).

Sediment samples were taken for Cu analysis at the end of the experiment. First, we removed the excess seawater from the sand with a capillary pipette that had been fire polished so the opening in the tip was smaller than the sand grains. Then we took six cores, 8 mm diameter, 1 cm deep, from the central area of each cup. The cores were split in half horizontally into a 'surface' and 'bottom' sample. The remaining live worms were removed from the cups, rinsed briefly (about 2 s) in deionized water, and frozen for body burden analysis. The remaining sand from each cup was sieved through a 1 mm Nytex* screen to remove the worm burrows. The sample referred to as 'worm burrow' is the mucous secreted by the worm, plus any attached sand grains. The remaining sand was sampled for Cu analysis and labelled 'residual'. All sediment samples were extracted by hot concentrated nitric acid. The extract was filtered, diluted to 25 ml with deionized water, and analyzed for Cu by atomic absorption analysis. Individual worms were freeze-dried, eluted in 10% nitric acid, and analyzed for Cu using a 2100 heated graphite atomizer coupled to a Perkin–Elmer atomic absorption spectrophotometer (Hoffman, personal communication).

We conducted a second experiment in an attempt to clarify the vertical distribution of Cu in the sand. Twelve cups of sand (1 cm deep) were exposed to a nominal concentration of 0.18 mg l^{-1} Cu for 28 days. The sand had been treated in a muffle furnace as described above. Ten cores were taken (as described above) from each cup on day 28. The cores were split in half horizontally and the bottom half labelled section D. The top half was split in half again and the bottom portion (one-quarter of total core) labelled section C. The top one-quarter of the core was divided into two equal sections, B and A, with A being the surface one-eighth of the core. Similar sections of the cores from each cup were pooled and analyzed for Cu.

The sediment data and body burden data were analyzed statistically by one-way analysis of variance. When significant differences were found, individual comparisons were made by partitioning the sums of squares (Snedecor & Cochran, 1967). All reported differences are significant at $\alpha = 0.05$ unless otherwise stated.

RESULTS

Actual Cu concentrations measured in each exposure box were 0.04 ± 0.01, 0.06 ± 0.01, 0.10 ± 0.015, 0.16 ± 0.02, and 0.26 ± 0.024 mg l^{-1} during the experiment. Natural background level of Cu in the seawater was 0.002 ± 0.0002 mg l^{-1}.

Neanthes arenaceodentata in the sand were less affected by Cu than those without sand (Fig. 1). The control mortality was similar for both treatments, 10% with sand, 20% without sand (one death probably due to injury from the other worm in the cup). Mortality at 0.04 mg l^{-1} Cu on day 28 was 10% with sand and 20% without sand. The LC50 values (Table 1) also show the difference in response between the

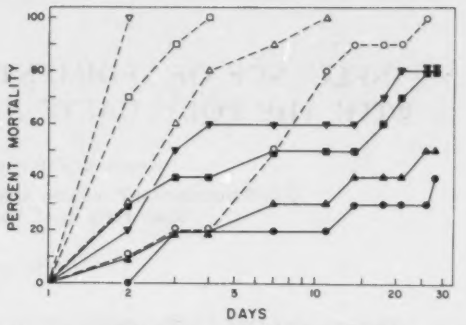


Fig. 1. Accumulative per cent mortality of adult male *Neanthes arenaceodentata* exposed to Cu in sea water with and without sand. Without sand: ▽——▽ 0.26 mg l^{-1} Cu, □——□ 0.16 mg l^{-1} Cu, △——△ 0.10 mg l^{-1} Cu, ○——○ 0.06 mg l^{-1} Cu. With sand: ▼——▼ 0.26 mg l^{-1} Cu, ■——■ 0.16 mg l^{-1} Cu, ▲——▲ 0.10 mg l^{-1} Cu, ●——● 0.06 mg l^{-1} Cu.

worms in sand and those without sand. Toxicity thresholds (incipient LC50), estimated graphically in Fig. 2, are about 0.04 mg l^{-1} and 0.10 mg l^{-1} Cu, without and with sand, respectively. However, the shape of the toxicity curve with sand is irregular. It appears to asymptote at 0.18 mg l^{-1}, with no further mortality between days 7 and 14. Then additional mortality occurred between days 14 and 28 resulting in a second asymptote at 0.10 mg l^{-1}. The experiment was not continued long enough to determine whether 0.10 mg l^{-1} was actually the toxicity threshold.

The average body burdens of Cu on *N. arenaceodentata* after a 28 day exposure are given in Table 2. One-way analysis of variance showed significant differences in the Cu concentrations in the worms from various treatments. Comparisons made on the control worms indicated that there was no significant difference between Cu concentrations of worms with and without sand. Comparisons made on the worms exposed to 0.04 mg l^{-1} Cu showed that the Cu concentrations of those in sand were significantly lower ($\alpha = 0.01$) than those without sand. The Cu concentrations of worms exposed to 0.06 mg l^{-1} with sand were significantly lower than those exposed to 0.04 mg l^{-1} without sand.

The body burdens of the *N. arenaceodentata* exposed to Cu in the presence of sand were greater when exposed to higher concentrations of Cu, up to the observed toxicity threshold (0.10 mg l^{-1}). There

Table 1. LC50 values (mg l^{-1}) for *Neanthes arenaceodentata* exposed to 0.04, 0.06, 0.10, 0.16 and 0.26 mg l^{-1} Cu in sea water with and without sand

Day	No sand	Sand
4	0.077	0.200
7	0.060	0.160
14	0.048	0.160
28	0.044	0.100

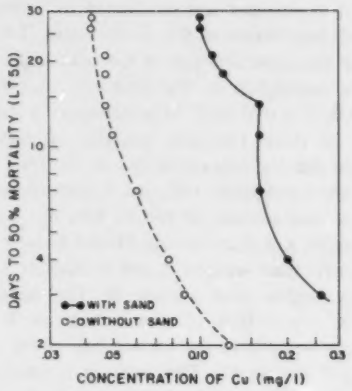


Fig. 2. Relationship between concentration of Cu and time to 50% mortality of *Neanthes arenaceodentata* with and without sand.

was a significant difference between the body burdens of worms exposed to control, 0.04, and 0.06 mg l^{-1} Cu with sand. Body burdens of worms in sand exposed to Cu concentrations at and above the toxicity threshold were variable, with no significant differences in body burdens between the 0.10, 0.16 and 0.26 mg l^{-1} Cu exposures. Copper concentrations of worms in sand exposed to 0.10 and 0.26 mg l^{-1} were not significantly different than those exposed to 0.06 mg l^{-1}.

Table 3 shows the extent of the adsorption of copper by the various sand samples. One-way analysis of variance of the six surface samples showed a significant difference in the Cu concentration of these samples. Comparisons indicated that the Cu concentrations of the surface samples exposed to 0.04 and 0.06 mg l^{-1} Cu were the same, but the surface samples exposed to all other concentrations were significantly

Table 2. Average concentration of Cu (μg g^{-1} dry wt) in *Neanthes arenaceodentata* after a 28 day exposure to 0.04 mg l^{-1} Cu in sea water with and without sand and a 28 day exposure to 0.06, 0.10, 0.16 and 0.26 mg l^{-1} Cu with sand

Exposure	Mean Cu concentration of worms	S.D.	Number of worms
Control, no sand	86	27	8
Control, sand	65	19	9
0.04, no sand	590	180	7
0.04, sand	287	117	9
0.06, sand	450	160	6
0.10, sand	309	149	5
0.16, sand	159	13	2
0.26, sand	258	156	2

Table 3. Concentration of Cu (μg g^{-1} dry wt) in sand samples after a 28 day exposure to 0.04, 0.06, 0.10, 0.16 and 0.26 mg l^{-1} Cu in the sea water

Concentration Cu in sea water (mg l^{-1})		Sand sample: Surface	Bottom	Residual	Burrow
Control (0.002)	$\bar{x}$	4.4	4.3	4.2	8.6
	S.D.	0.6	0.4	1.4	3.9
	n	5	5	5	5
0.04	$\bar{x}$	6.8	5.2	5.6	13.4
	S.D.	1.1	1.2	0.3	5.3
	n	5	5	5	5
0.06	$\bar{x}$	7.1	4.6	5.9	20.8
	S.D.	0.7	1.0	0.2	14.2
	n	5	5	5	5
0.10	$\bar{x}$	9.3	4.2	6.6	13.5
	S.D.	0.5	0.4	2.3	3.5
	n	4	4	4	4
0.16	$\bar{x}$	10.9	4.1	8.3	14.5
	S.D.	0.2	0.4	1.4	0.7
	n	2	2	2	2
0.26	$\bar{x}$	17.0	5.2	10.4	22.6
	S.D.	1.8	0.2	0.4	4.5
	n	2	2	2	2

$\bar{x}$ = mean, S.D. = standard deviation, *n* = number of samples.

different from each other. The Cu concentration in the surface sand samples was greater in the boxes with the higher concentrations of Cu in sea water. The regression of Cu concentration in surface sand samples on Cu concentration in sea water gave the following linear equation: $y = 4.47 + 46.53x$, with $r^2 = 0.94$. One-way analysis of variance on the six bottom samples indicated that the concentration of Cu in these samples was not significantly different. Differences in the Cu concentrations of the surface and bottom samples indicate that only the surface half (0.5 cm) of the sand adsorbed Cu. Copper concentrations for the residual samples, which were samples of the total depth of the sand, approximate the mean of the surface and bottom values as would be expected. Preliminary experiments showed that the Cu in the sea water remaining in the sand samples, contributed less than 1% of the Cu measured in the sand samples, even for samples taken from the highest exposure concentration, 0.26 mg l^{-1} Cu. Therefore, the Cu measured in the sand samples is the metal adsorbed to the sand.

One-way analysis of variance on the six burrow samples indicated no significant differences between exposures. The great variability in Cu concentration measured in the burrow samples might explain the lack of a statistically significant difference. This variability may be due to the different ratios of sand to mucous in each sample. One-way analysis of variance and comparisons done on the four samples (surface, bottom, burrow, residual) for each exposure indicate that the burrow sample at each exposure was significantly higher than the corresponding surface, bottom and residual samples. One exception was the 0.26 mg l^{-1} burrow sample which was higher than the surface sample only at the 8% level ($\alpha = 0.08$) of significance. Copper measured in burrow samples could be Cu associated with the mucous, the sand attached to the mucous, or both.

A budget for Cu in this experiment shows that the total amount of Cu adsorbed by the sand compared to the total amount of Cu added to the system in 28 days was small. The percentages of Cu adsorbed to sand were calculated to be 1.3% for 0.4 mg l^{-1}, 0.8% for 0.06 mg l^{-1}, 0.7% for 0.10 mg l^{-1}, 0.5% for 0.16 mg l^{-1}, and 0.5% for 0.26 mg l^{-1}.

A second experiment was conducted to determine the vertical distribution of Cu in the sand. Table 4 shows that the concentration of Cu was highest in the surface one-eighth of the core (A) after being exposed to 0.17 $\pm$ 0.02 mg l^{-1} Cu measured in the sea water for 28 days. One-way analysis of variance showed that the Cu concentrations in the four core sections were significantly different. Comparisons indicated that core sections D and C were not significantly different, but that sections D and C were significantly lower than sections A and B. Section A was significantly higher than section B. The weighted average, (2C + A + B)/4, of core sections A, B and C, which corresponds to the surface half of the core, is 11.9 μg g^{-1} dry wt Cu. This average is similar to the Cu concentration (10.9 μg g^{-1}) measured in the 0.16 mg l^{-1} Cu exposed surface core from the first experiment.

DISCUSSION

This study demonstrates that the presence of sand in a polychaete bioassay will increase the LC50 values for both short-term (4 and 7 days) and long-term (28 days) assays with Cu as the toxicant. However, the experiment was not carried out long enough to insure that the toxicity thresholds of the worms in sand had been reached. Further experimentation is needed to compare the toxicity thresholds of assays conducted with and without sand.

Several explanations are possible for the observed differences in LC50 values for *N. arenaceodentata* assayed with and without sand. The adsorption of Cu by the sand may cause a localized reduction of the concentration of Cu in the water immediately over the surface of the sand and circulating through the burrows. Thus, the worms in the sand would have been exposed to less Cu. For example, the worms exposed to 0.04 mg l^{-1} Cu without sand and 0.6 mg l^{-1} Cu with sand have similar mortality, 30 and 40%, respectively for day 28. However, the body burdens of the worms exposed to these two treatments for 28 days are significantly different, 590 μg g^{-1} Cu for 0.04 mg l^{-1} Cu exposure without sand and 450 μg g^{-1} Cu for 0.06 mg l^{-1} Cu exposure with sand. These differences in body burdens suggest

Table 4. Concentration of Cu (μg g^{-1} dry wt) in core sections of sand after a 28 day exposure to 0.17 mg l^{-1} Cu in the sea water

Core section	Mean Cu conc. μg g^{-1}	Standard deviation	Number of samples
A	28.6	5.7	12
B	8.7	1.5	12
C	5.1	0.8	12
D	4.7	0.4	12

A = surface to 1.25 mm deep, B = 1.25 to 2.5 mm deep, C = 2.5 to 5.0 mm deep, D = 5.0 to 10.0 mm deep.

that the worms in the sand may 'regulate' the Cu better, perhaps by controlling (slowing down) the flow of water circulated through the burrows, thus, being exposed to less Cu, or by being able to excrete Cu from the body more effectively. Wells & Dales (1951) have shown that polychaetes exhibit species specific patterns of circulating water through their burrows and that these patterns are altered when stressed. In the study of the uptake of cadmium by *Mya arenaria* with and without sediment conducted by Jackim *et al.* (1977), preliminary experiments indicated that the bivalve filtered more slowly when in sediment. Our data, though preliminary, suggest that both the Cu concentration and the response of the worms are influenced by the presence of sand.

The pattern of the Cu concentration in the tissues of *N. arenaceodentata* exposed in sediment is comparable to what McLusky & Phillips (1975) found with the polychaete *Phyllodoce maculata* even though the methods for handling the worms for analysis were different. McLusky & Phillips pooled specimens at each concentration. Only live worms were pooled from sublethal levels, while at lethal concentrations, live and dead animals were pooled. We analyzed *N. arenaceodentata* individually and used only live worms. McLusky & Phillips found that the concentration of Cu in the tissues of *P. maculata* were greater when exposed to higher Cu concentrations in the sea water, up to the toxicity threshold (0.08 mg l^{-1}). Body burdens of *P. maculata* exposed to Cu concentrations above the toxicity threshold were variable, and lower than the body burdens of worms exposed to the three highest sublethal concentrations. McLusky & Phillips suggested that it was not the amount of Cu in the tissues which resulted in death, but perhaps the rate of uptake which causes death. Although the trend is similar in this study, the Cu concentrations of *N. arenaceodentata* cannot be used to support McLusky's hypothesis because the Cu concentrations of the dead worms were not determined. The low and variable Cu concentrations of the few *N. arenaceodentata* left alive after 28 day exposure to lethal concentrations may simply represent biological variability in this species.

The linear relationship of the concentration of Cu in the sand to the concentration of Cu in the sea water suggests that the Cu measured in sand at all the exposure concentrations was metal adsorbed to the sand. If the copper in the sand from the two highest concentrations was metal precipitated, a curvilinear relationship would be expected.

In conclusion, the presence of sand does affect the results, both LC50 values and body burdens, of a bioassay exposing polychaetes to Cu. Further research is needed with various sediment types of different adsorptive characteristics, and with different toxicants, to fully evaluate the impact of sediment on polychaete bioassay results.

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